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14. ABSTRACT

The goal of this research was to investigate efficacy and mechanisms of H5BVIFN-β, a novel immunotherapeutic agent, against prostate cancer in animal models. The objectives in year 2 were to determine efficacy of a mixture of irradiated TRAMP-L5 mouse prostate cancer cells and lyophilized H5BVIFN-β in regressing orthotopic tumors of TRAMP-L5 cells and to study effects of intratumoral injection of H5BVIFN-β on expression of immune stimulatory cytokine in tumors. We found that subcutaneous injections of irradiated TRAMP-L5 cells, recombinant IFN-β, or H5BVIFN-β, partially inhibited orthotopic growth of TRAMP-L5 cells in syngeneic mice. An additive inhibitory effect was observed in mice injected with a mixture of irradiated TRAMP-L5 cells and H5BVIFN-β. However, none of the therapies was able to eradicate orthotopic tumors. Intratumoral delivery of H5BVIFN-β was not able to enhance expression of immune-stimulatory cytokines interleukin (IL)-2, IL-12, or interferon-γ.

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Table of Contents

INTRODUCTION

The specific aims of this research are unaltered from the original proposal. They remain to be investigating efficacy of our novel immunotherapy system in the treatment of occult prostate cancer and mechanisms by which this therapy suppresses tumor growth. The task for the first year is to determine the efficacy of the therapy.

In the year 1 of this research, we found that TRAMP-L5 tumors grow too fast to be eradicated and proposed to replace TRAMP-L5 cells with TRAMP-C2 cells, which was granted. To test the tumorigenicity of TRAMP-C2 cells, we injected 10^6/mouse of the cells into the subcutis and the prostate of C57BL/6 mice (two times each) and found that the tumor-take rate was about 50% in both models. These experiments concluded that TRAMP-C2 cells are not suitable for carrying out studies proposed. We then tested effects of our novel therapy system on growth of TRAMP-C2RE3 tumors, which, also derived from TRAMP-C2 and grow slightly slower than TRAMP-L5, have been used in one of our recent publications in study adenoviral vector-mediated IFN-β gene therapy (1). We found that efficacy of H5BVIFN-β therapy against TRAMP-C2RE3 tumors (Fig. 1 and 2) was similar to that against TRAMP-L5 tumors. Therefore, we continued our studies using TRAMP-L5 cells as proposed in our original proposal and plan to use the same model during the studies of year 3.

PROGRESS REPORT BODY

1. To investigate efficacy of intratumoral injection of H5BVIFN-β in suppressing growth and metastasis of TRAMP-C2RE3 tumors in immune-competent syngeneic mice

a. Doses of H5BVIFN-β needed to eradicate orthotopic TRAMP-C2RE3 tumors.

TRAMP-C2RE3 cells (10^5/mouse) were injected into the prostate of male C57BL/6 mice. Seven days after the orthotopic implantation, mice were intralesionally injected with 0.5, 1, or 2 units of H5BVIFN-β (1 unit of H5BVIFN-β contains 2×10^4 units of IFN-β and 10^6 lyophilized H5 insect cells). Tumors injected with PBS, 4×10^4 units of IFN-β or 2×10^6 lyophilized H5 cells served as controls. The experiments were terminated 3 weeks after the therapies. The prostate tumors were removed and weighed. As shown in Fig. 1, a single intratumoral injection of H5BVIFN-β inhibited tumor

growth in a dose-dependent manner. Injection of H5 or IFN-β did not significantly alter growth of TRAMP-C2RE3 tumors. Treatment with 2 units of H5BVIFN-β suppressed tumor growth by approximately 70%. However, similar to that in treating TRAMP-L5 tumors, this therapy was not able to eradicate tumors in any treated mice.

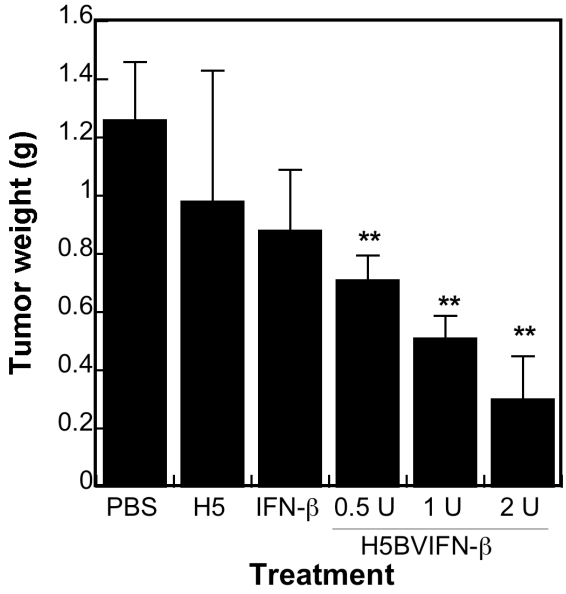

Fig. 1. Efficacy of H5BVIFN-β therapy against orthotopic TRAMP-C2RE3 tumors
**, p<0.01, in comparison with tumors injected with PBS.

b. Effects of H5BVIFN-β therapy on the survival of tumor-bearing mice.

TRAMP-C2RE3 cells were inoculated into the prostates of C57BL/6 mice. Seven days later, tumor-bearing mice were divided into 3 groups and treated by intratumoral injection of PBS, 2 x 10^6 lyophilized H5 cells, or 2 units of H5BVIFN-β. Mice were monitored daily and sacrificed only when they became moribund. Effects of the treatments on the survival of tumor-bearing mice were analyzed by Kaplan-Meier method. Median survival times for mice injected with PBS, H5 cells, IFN-β, and H5BVIFN-β were 32, 31.5, and 39 days, respectively. Only the injection of H5BVIFN-β significantly prolonged survival of tumor-bearing mice ($p<0.01$ in comparison with those injected with PBS). Data shown in Fig. 2 are results pooled from 2 independent experiments (with 8 mice/group).

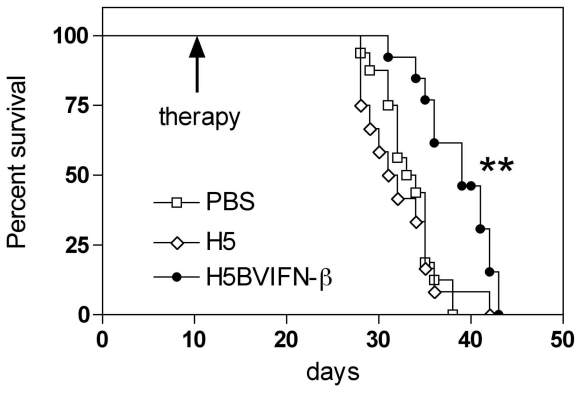

Fig. 2. Effects of H5BVIFN-β therapy on survival of mice carrying orthotopic TRAMP-C2RE3 tumors.
**, p < 0.01 in comparison with tumors injected with PBS.

2. To investigate efficacy of subcutaneous injection of a mixture of irradiated TRAMP-L5 cells and H5BVIFN-β in suppressing growth of TRAMP-L5 tumors in the prostate of C57BL6 mice

a. Dose-dependent effects of irradiated TRAMP-L5 cells

Majority of TRAMP-L5 cells irradiated at a dose of 10,000 rads were viable for up to 5 days in tissue culture but lost their tumorigenicity in mice (data not shown). We used cells irradiated at 10,000 rads as tumor antigen in the study. TRAMP-L5 cells (10^5/mouse) will be inoculated into the prostates of C57BL/6 mice. Three days later, mice were subcutaneously injected with a mixture of 1, 2, 5, 10, or 20 x 10^6 irradiated TRAMP-L5 cells and 2 units of H5BVIFN-β. Mice injected with PBS, 10 x 10^6 irradiated TRAMP-L5 cells, or H5BVIFN-β alone served as controls. The injections were repeated twice a week for three weeks. The experiment was terminated when mice in any group became moribund.

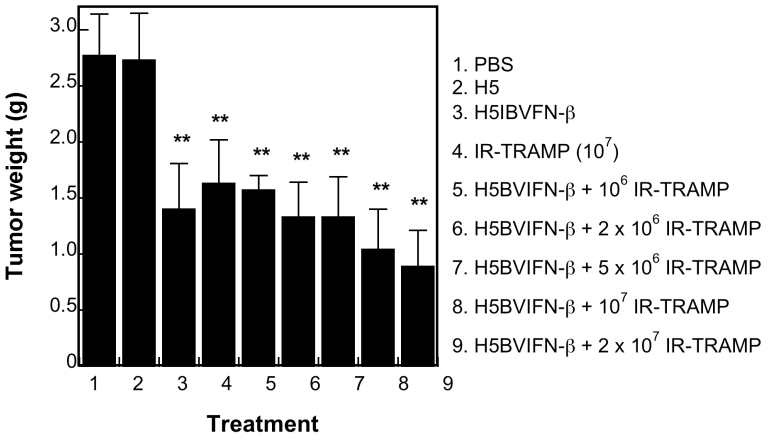

Fig. 3. Effects of a mixture of irradiated TRAMP-L5 and H5BVIFN-β on orthotopic growth of TRAMP-L5 tumors
**, $p < 0.01$ in comparison with mice treated with PBS or H5 cells.

As shown in Fig. 3, the therapy of subcutaneous injection with the mixture inhibited growth of TRAMP-L5 tumors in a dose-dependent manner. In mice injected with a mixture of 2 units of H5BVIFN-β and 20 x 10^6 irradiated TRAMP-L5 cells, growth of the orthotopic TRAMP-L5 tumors was inhibited by approximately 60%. However, none of the mice were cured of primary tumors in the prostates. The injection of irradiated TRAMP-L5 cells also retarded growth of orthotopic tumors (group 4 in Fig. 3), suggesting the antigenic effects of the cells. Surprisingly, the injection of H5BVIFN-β alone (group 3 in Fig. 3) also inhibited tumor growth.

b. Tumor specificity and effects of circulating IFN-β

To assess the specificity of the therapy of the mixture of irradiated TRAMP-L5 and H5BVIFN-β, mice were injected with a mixture of H5BVIFN-β and 10 x 10^6 irradiated 3LL Lewis lung carcinoma cells. The injections were repeated twice a week for three weeks. The experiment was terminated when mice in any group became moribund. As shown in Fig. 4, subcutaneous injection of irradiated 3LL cells did not affect growth of orthotopic implanted TRAMP-L5 cells. In fact, effects of H5BVIFN-β on growth of TRAMP-L5 cells was reduced when it was mixed with irradiated 3LL cells, possibly absorption of IFN-β by the irradiated 3LL cell in the mixture. To explore potential role of circulating IFN-β on orthotopic growth of TRAMP-L5 tumors, one group of mice were injected with 4 x 10^4 units of recombinant IFN-β. The injections were repeated twice a week for three weeks. We found that the repeated injection of IFN-β also suppressed growth of TRAMP-L5 tumors in the prostate.

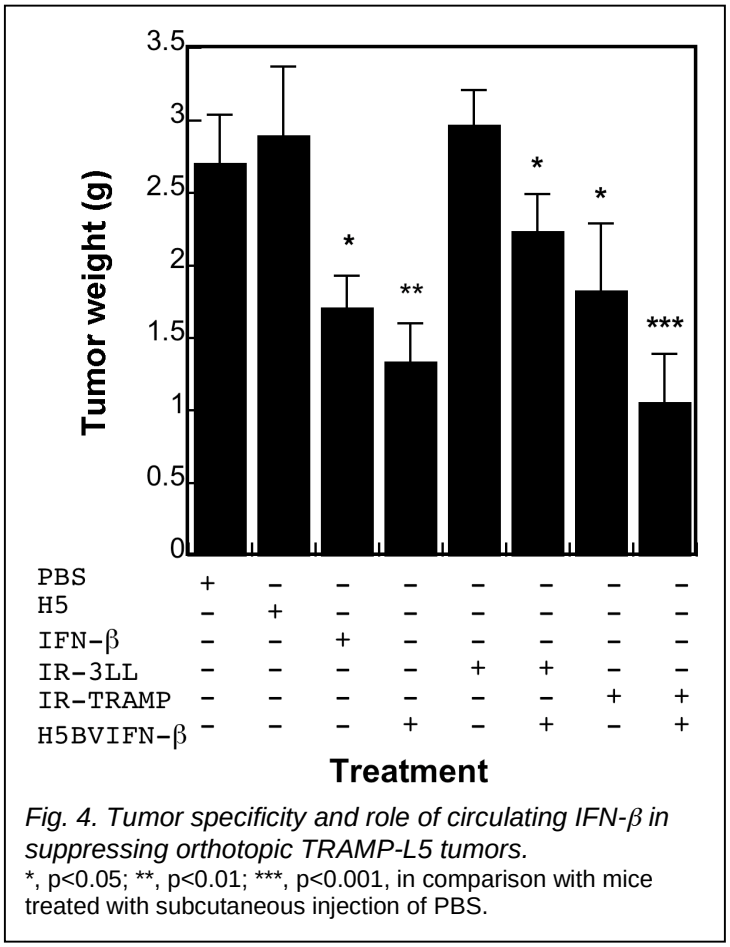

Fig. 4. Tumor specificity and role of circulating IFN-β in suppressing orthotopic TRAMP-L5 tumors.
*, p<0.05; **, p<0.01; ***, p<0.001, in comparison with mice treated with subcutaneous injection of PBS.

c. The minimal numbers of injection necessary for the therapy and efficacy of the therapy on large tumors vs. small tumors.

Since we were not able to eradicate orthotopic TRAMP-L5 tumors by using the mixture of irradiated TRAMP-L5 cells and H5BVIFN-β, the experiments determining the

minimal numbers of injection necessary for the therapy and efficacy of the therapy on large tumors vs. small tumors were not performed.

3. To investigate molecular events responsible for the therapeutic effects of H5BVIFN-β

To determine whether H5BVIFN-β induces IL-2, IL-12 and IFN-gamma and downregulates TGF-β1 in orthotopic TRAMP-L5 tumors.

Orthotopic TRAMP-L5 tumors in C57BL/6 mice were injected with 2 units of H5BVIFN-β. Tumors injected with PBS, 2 x 10^6 lyophilized H5 cells, or 4 x 10^4 units of recombinant murine IFN-β served as controls. On days 3 and 7 after the treatment, mice were sacrificed and tumors were sampled for in vitro analyses to determine IL-2 (Fig. 5A), IL-12 (Fig. 5B), and IFN-γ (Fig. 5C). Data shown in Fig. 6 indicate that H5BVIFN-β failed to enhance the expression of any one of the immune stimulatory cytokines. Expression of TGF-β1 and inducible nitric oxide synthase in control and treated tumors are currently examination now.

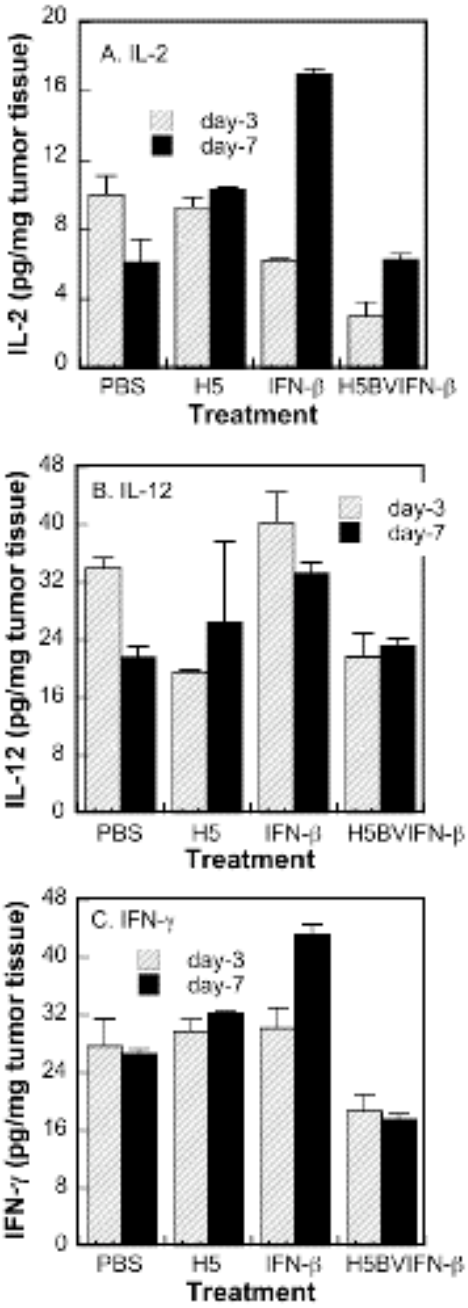

Fig. 5. H5BVIFN-β therapy on the expression of immune cytokines in TRAMP-L5 tumors

4. Recommended additional experiments in the next year

H5BVIFN-β therapy inhibited tumor growth and prolonged survival of tumor-

bearing mice. However, it could not mount a potent tumor-specific immune response to eradicate primary tumors. Data in Fig. 5 show that it failed to enhance the expression of immune-stimulatory cytokines necessary for the induction of tumor-specific immunity. These data suggest that inhibition of tumor growth by H5BVIFN-β was mediated by some local mechanisms. Cell replication and death are the two parameters that determine tumor growth rate. We, therefore, propose to investigate expression of proliferating cell nuclear antigen (PCNA), which is expressed mainly in the late G1 and M phase of the cell cycle and indicates cell replication (2), by immunohistochemical staining and apoptosis by TUNEL staining (1,3), respectively. On day 3 and 7 after the intralesional injection of PBS, H5, IFN-β, or H5BVIFN-β, tumors will be sampled for in vitro analyses. Since IFN-β is a potent antiangiogenic molecule, we propose to further study whether H5BVIFN-β therapy alters angiogenesis in the tumor lesions. Tumors will be injected with PBS, H5, IFN-β, or H5BVIFN-β. On day 5 after the therapy, tumors will be sampled for immunohistochemical staining using an antibody against CD31, which is expressed on microvessel endothelial cells.

KEY ACCOMPLISHMENT

We have completed research proposed in the task 2 of the SOW and started research on the task 3 of the SOW.

REPORTABLE OUTCOMES

No reportable outcomes have yet to arise from this project.

CONCLUSIONS

1. We have confirmed, in TRAMP-C2RE3 tumor model, that intratumoral injection of H5BVIFN-β significantly inhibited orthotopic growth of mouse prostate cancer cells and that the therapy moderately but significantly prolonged the survival of tumor-bearing mice.
2. The HBVIFN-β therapy could not eradicate TRAMP-C2RE3 orthotopic tumors.

3. Repeated subcutaneous injection (twice a week) of irradiated TRAMP-L5 cells, IFN-β, or H5BVIFN-β could inhibit growth of orthotopic TRAMPL5 tumors.
4. Intratumoral injection of H5BVIFN-β could not enhance or upregulate immune-stimulatory cytokines, IL-2, IL-12, and IFN-γ in orthotopic TRAMP-L5 tumors, which could be the reason that this therapy failed to mount a strong tumor-specific immune response necessary for the eradication of tumors.
5. Our data suggest that intratumoral injection of H5BVIFN-β could be a potential novel therapy for human prostate cancer.

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APPENDICES

NONE.